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An Efficient Synthesis of Mimetics of Neamine for RNA Recognition

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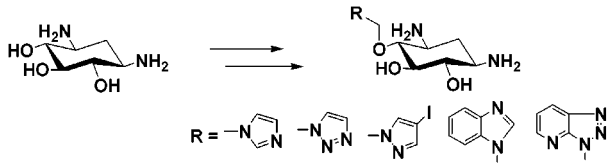
ABSTRACT



As mimetics of neamine, several 4-heterocyclic 2-deoxystreptamine derivatives were chemically synthesized for RNA recognition. Conversion of 4-methylthiomethyl-5,6-di-*O*-acetyl-diazido-2-deoxystreptamine to the 4-chloromethyl derivative followed by reactions with different nuclophilic reagents gave the 4-heterocyclic 2-deoxystreptamine derivatives in satisfactory yields.

Several aminoglycosides are known to interact with RNA and interfere with its function.[1] For example, neomycin B induces translational misreading, most likely as a result of an interaction with 16S *r*RNA in the A site of the ribosome.[2] Direct use of neomycin B as a drug, however, has been discouraged as a result of its high toxicity, instability, and poor oral bioavailability.[3] The structures of the aminoglycosides are synthetically challenging and do not lend themselves to the rapid preparation necessary for a medicinal chemistry program.[4] Therefore, it is highly desirable to synthesize mimetics of aminoglycosides that are smaller, simpler structures and retain the activity of the larger parent structures.

Most naturally occurring aminoglycosides share a common pseudodisaccharide known as neamine. Therefore, mimetics of neamine would be an ideal starting point for the synthesis of new potential antibiotics. In this communication, we report an efficient strategy for the synthesis of mimetics of neamine.

Computer modeling studies indicated that if the A ring of neomycin B was replaced by a benzyl group, then the resulting compound would keep the conformation of neomycin B.[5] We assumed that the conformation of neomycin B plays a very important role in its RNA binding affinity and specificity. So, we chose the 4-heterocyclic 2-deoxystreptamine derivatives as target compounds. Two approaches could be used for synthesis of target compounds (Scheme 1).

One approach is simple alkylation of diacetylated 2-deoxystreptamine derivative **1** with different Het-CH_2X (X = Cl, Br, $CNHCCl_3$).[6] By a second approach, 4-chloromethyl-5,6-di-*O*-acetyl-diazido-2-deoxystreptamine (**2**) can be coupled with different heterocycles such as HetYH (Y = N, S) to provide the heterocyclic 2-deoxystreptamine derivatives.

Alkylation of compound **1** with several Het-CH_2X (X = Cl, Br) under different base conditions failed as a result of

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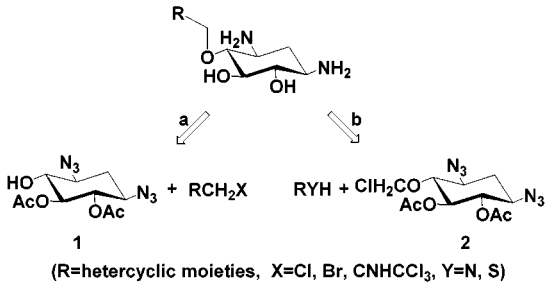
Scheme 1



the base sensitivity of acetyl groups. We were not able to isolate any desired products from the reaction mixture. Several substituted benzyl trichloroacetimidates could alkylate the 4-hydroxyl group of compound **1** in the presence of triflic acid; however, the yields were very low.

As an alternative approach, compound **1** was treated with $CH_2(OMe)_2$ in the presence of P_2O_5 to give the MOM-protected 2-deoxystreptamine derivative **3**. Conversion of the MOM ether group into RCH_2Cl by reaction with BCl_3 in dichloromethane failed, and the major product was the MOM ether cleaved compound. Attempts to couple compound **3** with different HetYH (Y = N, S) agents directly, according to known procedures,[7] gave only trace amounts of products.

Using the Pummerer rearrangement,[8] the MTM ether group could be introduced at the 4-position of compound **1** under neutral conditions. Treatment of **1** with excess DMSO/Ac_2O/AcOH at room temperature for 48 h gave the MTM-protected 2-deoxystreptamine derivative **4** in 78% yield.

Conversion of compound **4** to the key intermediate **2** was achieved by treatment with excess SO_2Cl_2 in CH_2Cl_2.

Compound **2** was then coupled with several nucleophilic reagents under different conditions to afford the corresponding 4-heterocyclic 2-deoxystreptamine derivatives in satisfactory yields (Table 1).

Imidazole, benzimidazole, and 2-(trifluoromethyl)-benzimidazole were acetylated with acetic anhydride, and the resulting compounds were coupled with **2** in the mixture of dichoromethane and acetonitrile to afford the products. After deacetylation with sodium methoxide in methanol, compounds **5**−**7** were isolated.

1,2,3-Triazole, 1,3,4-triazole, and 4-iodopyrazole were first treated with 1.2 equiv of sodium hydride in acetonitrile to provide the corresponding sodium salts and then coupled with compound **2**. After work up and deacetylation, products **8**−**10** were isolated in high yields.

The same procedure was applied to 2-mercaptopyrimidine, and the sulfur-linked 4-heterocyclic 2-deoxystreptamine derivative **11** was obtained. In the case of 4-(trifluoromethyl)-2-pyridinethiol, decomposition occurred when methanolic sodium methoxide was used for deacetylation. However, deacetylation in methanol/NH_4OH (4:1) gave the product **12**.

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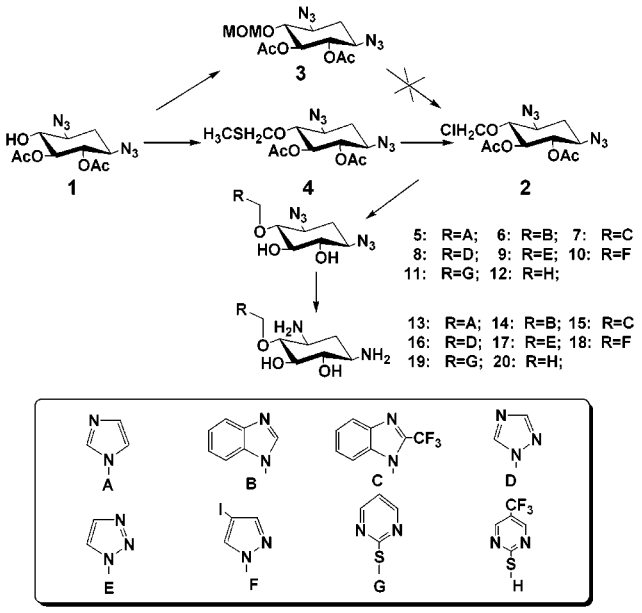
Table 1

entry	RH	reaction solvent	products	yield (%)
1	imidazole	CH_2Cl_2	**5**	86
2	benzimidazole	CH_2Cl_2	**6**	81
3	2-(trifluoromethyl)-benzimidazole	CH_2Cl_2/CH_3CN (1:1)	**7**	76
4	1,2,3-triazole	CH_3CN	**8**	93
5	1,3,4-triazole	CH_3CN	**9**	95
6	4-iodopyrazole	CH_3CN	**10**	97
	2-mercaptopyrimidine	CH_3CN/DMF (4:1)	**11**	84
8	4-(trifluoromethyl)-2-pyridinethiol	CH_3CN/DMF (4:1)	**12**	89
9	1*H*-1,2,3-triazole-[4,5]-pyridine	CH_3CN/DMF (2:1)	**21**, **22**, **23**	92[a]
10	3-(trifluoromethyl)-pyrazole	CH_3CN	**24**, **35**	90[a]
11	6-bromopurine	CH_3CN/DMF (1:1)	**31**, **32**	95[a]

[a] The yields were calculated based on the consumption of compound **2**.

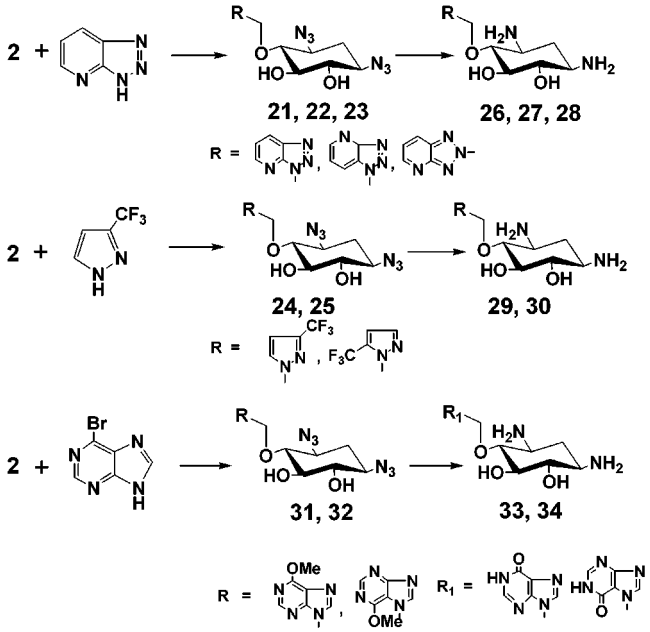
After deprotection of compounds **5**−**12** with Me_3P/THF/H_2O, the neamine mimetics **13**−**20** were obtained (Scheme 2).

Scheme 2



The strategy described above was used for the synthesis of small libraries of 4-heterocyclic 2-deoxystreptamine (Scheme 3). Treatment of 1*H*-1,2,3-triazolo-[4,5]-pyridine with 1.2 equiv of sodium hydride in the mixture of CH_3-CN/DMF at room temperature for 1 h, followed by reaction with compound **2**, gave a mixture of three products. After deacetylation, compounds **21**−**23** were isolated as a mixture in a ca. 1:1:0.5 ratio. In a similar way, a mixture of

Scheme 3



compounds **24** and **25** (2:1) was synthesized starting from 3-(trifluoromethyl)pyrazole. After deprotection, two libraries containing compounds **26**−**30** were obtained.

Using the same procedure, the coupling reaction of compound **2** with the sodium salt of 6-bromopurine gave a mixture of two products in a ca. 2:1 ratio. When this mixture was treated with sodium methoxide in methanol at room temperature, a mixture of compounds **31** and **32** was obtained. Reduction of the azido groups with Me_3P/THF/H_2O followed by treatment with 1 N HCl provided a mixture of compounds **33** and **34**.

Electrospray ionization mass spectrometry can be employed to determine solution-phase dissociation constants of the RNA-ligand complex on the basis of gas-phase measurements of the ratio of free and bound RNA target.[9] As mimetics of neamine, the final products were used for their 16S RNA binding studies. By using this method, we are able to evaluate the binding affinities of the final products for a 27-mer RNA representing the 16S A-site. Compounds were screened against the 16S A-site at equal concentrations in separate experiments, and the resulting estimated dissociation constants (based on a 1 point K_d determination) are reported in Table 2.

Table 2. Dissociation Constants of 16S-Ligand Complexes Based on Gas-Phase Measurements of the Ratio of Free and Bound RNA Targets[a]

compounds	dissociation constants (μM)
13	275
14	667
15	439
16	378
17	355
18	682
19	488
20	1079
26, 27, 28	554[b]
29, 30	441[b]
33, 34	100[b]
Neamine	24

[a] Ligands, 75 μM; target RNA, (2.5 μM) [b] These dissociation constants were measured for the mixture of two or three isomers.

4-Purine-2-deoxystreptamine derivatives **33** and **34** showed better binding affinities than other 4-heterocyclic 2-deoxystreptamine derivatives. On the basis of these results, the synthesis of more complex mimetics of aminoglycosides is in progress.

In conclusion, the synthetic strategy described here provides a very efficient method to synthesize heterocyclic carbohydrate derivatives for the biological screen.

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Supporting Information Available: NMR spectroscopic data for compounds **5**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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